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EXAMINER	
SAMSON, MARIA TERESA D	
ART UNIT	**PAPER NUMBER**
1638	

DATE MAILED: 03/11/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/725,829	CHYE ET AL.	
	Examiner	Art Unit	
	Maria Teresa Samson	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09-December 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-58* is/are pending in the application.

4a) Of the above claim(s) *2, 4-7 and 48* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 3, 8-47 and 49-58* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *01-January 2003* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *See search notes*.

DETAILED ACTION AND OFFICE ACTION SUMMARY FORM

Applicant's election with traverse of Group I, claims, 1-4, 8-47 and 49-58, in the reply filed on 09-December 2004 is acknowledged. The traversal is on the ground(s) that ten sequences constitute a reasonable number for examination purposes, and that up to ten independent and distinct nucleotide sequences will be examined in a single application without restriction. This is not found persuasive. One sequence is "up to ten" and because, given the database size and resource allocation at the USPTO, examination of more than one sequence would present a severe burden on USPTO resources.

Additionally, a protein is not obvious over the polynucleotide that encodes it and that the polynucleotide and the polypeptide are not related because the polynucleotide encodes the polypeptide. The polypeptide is not directly made from the DNA molecule that encodes it. While the nucleic acid sequence may provide researchers the amino acid sequence of the initially-translated protein, it does not allow them to accurately predict properties of the protein like K_m, temperature maximum, or even molecular weight of the processed protein. Additionally, the protein can be isolated from the natural source and characterized in detail without knowledge of the DNA that encodes it, and in fact, many proteins were isolated years before DNA cloning and sequencing were possible. Thus, the protein is not obvious over the nucleic acid that encodes it, and vice versa.

Furthermore, the claims are not limited to single nucleic acid sequences or amino acid sequences, but encompass a multitude of nucleic acid sequence variants encoding a multitude of amino acid sequences with varying properties.

The requirement is still deemed proper and is therefore made FINAL.

Claims 2, 4-7 and 48 and DNA encoding SEQ ID NO: 4 or hybridizing to SEQ ID NO: 3 are withdrawn from consideration as being drawn to non-elected inventions.

Claims 1, 3 and 8-47 and 49-58 are examined on the merits.

Specification

(A.) The drawings are objected to because figures 5-9 are too dark and no details can be made out. The petition filed under 37 CFR 1.84(a)(2) is acknowledged.

(B.) The oath or declaration is defective because the applicants have not signed the oath and declaration.

Claim Objections

Claims 8, 16, 24, 27, 30, 33, 36, 39, 49, 50 are objected to for reciting nonelected SEQ ID NOs.

Claims 12, 13, 14, 15, 17, 18, 20, 21, 22, 23, 25, 26, 28, 29, 31, 32, 34, 35, 37, 38, 40, 41, 42, 43, 44, 45, 51, 52, 56, 57, 58 are objected to because of the following informalities:

(A.) In claims 12, 13 and 14, a comma is missing after the number 11.

Similarly, in claim 15, a comma is missing after the number 14.

Similarly, in claims 17 and 18 a comma is missing after the number 16.

Similarly, in claims 20 and 21, a comma is missing after the number 19.

Similarly, in claim 22, a comma is missing after the number 17.

Similarly, in claim 23, a comma is missing after the number 20.

Similarly, in claim 25, a comma is missing after the number 24.

Similarly, in claim 26, a comma is missing after the number 24.

Similarly, in claims 28 and 29, a comma is missing after the number 27.

Similarly, in claims 31 and 32, a comma is missing after the number 30.

Similarly, in claims, 34 and 35, a comma is missing after the number 33.

Similarly, in claims 37 and 38, a comma is missing after the number 36.

Similarly, in claims 40 and 41, a comma is missing after the number 39.

Similarly, in claim 43, a comma is missing after the number 42.

Similarly, in claim 44, a comma is missing after the number 43.

Similarly, in claim 45, a comma is missing after the number 44.

Similarly, in claim 51, a comma is missing after the number 50.

Similarly, in claim 52, a comma is missing after the number 51.

Similarly, in claim 56, a comma is missing after the number 55.

Similarly, in claim 57, a comma is missing after the number 56.

Similarly, in claim 58, a comma is missing after the number 56.

(B.) Claim 42 and claims 43-45 dependent there on are objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim should refer to claims in an alternative form. See MPEP § 608.01(n). Examination of the claims does not relieve applicants of the requirement to correct the claim dependency.

(C.) Claim 23 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim, or amend the claim or to place the claim in proper dependent form, or rewrite the claim in independent form. Specifically:

(1.) Claim 23 recites the method of claim 20 wherein the endogenous proteinase activity is a trypsin-like activity or chymotrypsin-like activity. Claim 20 does not make reference to an endogenous proteinase activity. However, claim 22 makes a reference to

an endogenous proteinase activity. If the applicant intended for claim 23 to further limit claim 22, then Applicant is suggested to amend claim 22.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

(A.) Claims 46, 47, 53 and 54 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claims 46, 47, 53 and 54 contain vectors, pSa7 and pMLVHisP, subject matter that was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claim is directed to specific plasmids. Since the plasmids are essential to the claimed invention, they must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. If the plasmids are not so obtainable or available, a deposit of microorganism containing said plasmids may satisfy the requirements of 35 USC 112. The specification does not disclose a repeatable process to obtain the plasmids and it is not apparent if the plasmids are readily available to the public. Thus, a deposit is required for enablement purposes.

If the deposit is made under the terms of the Budapest Treaty, then an affidavit or declaration by Applicant, or a statement by an attorney of record over his or her signature and registration number, stating that the specific strain has been deposited under the Budapest Treaty and that the strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent, would satisfy the deposit requirement made herein.

If the deposit has not been made under the Budapest Treaty, then in order to certify that the deposit meets the criteria set forth in 37 C.F.R. 1.801-1.809, Applicant may provide assurance of compliance by an affidavit or declaration, or by a statement by an attorney of record over his or her signature and registration number, showing that

(a) during the pendency of this application, access to the invention will be afforded to the Commissioner upon request;
(b) all restrictions upon availability to the public will be irrevocably removed upon granting of the patent;
(c) the deposit will be maintained in a public depository for a period of 30 years or 5 years after the last request or for the enforceable life of the patent, whichever is longer;
(d) a test of the viability of the biological material at the time of deposit (see 37 CFR 1.807); and,
(e) the deposit will be replaced if it should ever become inviable.

In addition, the identifying information set forth in 37 CFR 1.809(d) should be added to the specification. See 37 CFR 1.801 - 1.809 [MPEP 2401-2411.05] for additional explanation of these requirements.

(B.) Claims 3, 11-15, 19-23, 27-29, 33-35, 39-45, 50-52, and 55-58 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claims contain subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claim is broadly drawn to an isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1 wherein the nucleotide sequence encodes a protein having proteinase inhibitor acitivty.

Applicant does not describe an isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1 wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity.

Applicant has not described any structural features of SEQ ID NO:1 that are essential for function and Applicant has not described if nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1 possesses the structural features that are essential for function. Furthermore, there is no functional description of an isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1. In addition, Applicant does not describe the sufficient structural elements of a representative number of nucleic acids that encode a proteinase inhibitor II.

Hence, Applicant has not, in fact, described an isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1 wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity within the full scope of the claims, therefore the specification fails to provide an adequate written description of the claimed genus.

Therefore, given the lack of written description in the specification with regard to the structural and functional characteristics of the claimed nucleic acids, it is not clear that Applicant was in possession of the claimed genus at the time this application was filed.

(C.) Claims 3, 11-15, 19-23, 27-29, 33-35, 39-47, and 50-58 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for an isolated proteinase inhibitor II nucleic acid molecule having a nucleotide sequence of

SEQ ID NO: 1 encoding SEQ ID NO: 2, method for producing a transformed plant comprising a polynucleotide that comprises the nucleotide sequence of SEQ ID NO: 1 encoding SEQ ID NO: 2 and selecting a transformed plant in which said nucleotide sequence is expressed, and endogenous protease activity of transformed plant is inhibited, does not reasonably provide enablement for an isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1, a method for producing a transformed plant comprising a polynucleotide that hybridizes to the nucleotide sequence of SEQ ID NO: 1 and selecting a transformed plant in which said nucleotide sequence is expressed and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity and endogenous proteinase activity of the transformed plant is inhibited, a method for inhibiting programmed cell death and senescence in transformed plant or plant part, a method for producing a heterologous protein in a plant comprising transforming a plant with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to SEQ ID NO: 1 and transforming the plant with a second polynucleotide that encodes a heterologous protein and isolating said heterlogous protein, a transformed plant produced by transforming a plant with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1 wherein the nucleotide sequence encodes a protein having proteinase inhibitor acitivity and selecting a transformed plant in which said polynucleotide is expressed, a transformed plant comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1, a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID

NO: 1, a recombinant cell comprising the recombinant vector. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are broadly drawn to an isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1, a method for producing a transformed plant comprising a polynucleotide that hybridizes to the nucleotide sequence of SEQ ID NO: 1 and selecting a transformed plant in which said nucleotide sequence is expressed and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity and endogenous proteinase activity of the transformed plant is inhibited, a method for inhibiting programmed cell death and senescence in transformed plant or plant part, a method for producing a heterologous protein in a plant comprising transforming a plant with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to SEQ ID NO: 1 and transforming the plant with a second polynucleotide that encodes a heterologous protein and isolating said heterlogous protein, a transformed plant produced by transforming a plant with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1 wherein the nucleotide sequence encodes a protein having proteinase inhibitor acitivity and selecting a transformed plant in which said polynucleotide is expressed, a transformed plant comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SE ID NO: 1, a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1, a recombinant cell comprising the recombinant vector. The specification does not

enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The instant specification, however, only provide guidance for cloning and DNA sequence analysis of the 5'-end of the SaPIN2b cDNA (page 36); expression patterns of SaPIN2a and SaPIN2b (page 45); localization of SaPIN2a and SaPIN2b mRNA and proteins in flowers (pages 45-46); immunogold labeling pf SaPIN2a and SaPIN2b in *S. americanum* ovuile (page 47); generation of transgenic of lettuce with pSa7 containing SaPIN2a cDNA (pages 40 and 47); Southern blot analysis of transgenic lettuce (page 48); expression of SaPIN2a mRNA in transgenic lettuce (page 49); trypsin and chymotrypsin inhibitory activities and endogenous trypsin- and chymotrypsin-like activity assays (pages 42 and 50); preliminary insect feeding assay with primary transgenic lettuce plants (pages 43 and 51); plasmid construction for plastid transformation of tobacco with plasmid pMLVHisP (pages 43 and 52) and screening of the plastid-transformed tobacco for integration of SaPIN2a cDNA (page 44).

The instant specification fails to provide guidance for an isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1, a method for producing a transformed plant comprising a polynucleotide that hybridizes to the nucleotide sequence of SEQ ID NO: 1 and selecting a transformed plant in which said nucleotide sequence is expressed and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity and endogenous proteinase activity of the transformed plant is inhibited, a method for inhibiting programmed cell death and senescence in transformed plant or plant part, a method for producing a heterologous protein in a plant comprising transforming a plant

with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to SEQ ID NO: 1 and transforming the plant with a second polynucleotide that encodes a heterologous protein and isolating said heterlogous protein, a transformed plant produced by transforming a plant with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1 wherein the nucleotide sequence encodes a protein having proteinase inhibitor acitivity and selecting a transformed plant in which said polynucleotide is expressed, a transformed plant comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1, a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1, a recombinant cell comprising the recombinant vector. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

In re Wands, 858F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988) lists eight considerations for determining whether or not undue experimentation would be necessary to practice an invention. These factors are: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples of the invention, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims.

The specification does not teach where to find or how to make the isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1.

Making substitutions in nucleic acid does not produce predictable results. Lazar et al (1988, Mol. Cell. Biol. 8:1247-1252) showed that the "conservative" substitution of glutamic acid for aspartic acid at position 47 reduced biological function of transforming growth factor alpha while "nonconservative" substitutions with alanine or asparagine had no effect (abstract). Similarly, Hill et al (1998, Biochem. Biophys. Res. Comm. 244:573-577) teach that when three histidines that are maintained in ADP-glucose pyrophosphorylase across several species are substituted with the "nonconservative" amino acid glutamine, there is little effect on enzyme activity, while the substitution of one of those histidines with the "conservative" amino acid arginine drastically reduced enzyme activity (see Table 1). In addition, the specification fails to provide any guidance with regard to which sequences that hybridize would encode a polypeptide having proteinase II activity.

Given the claim breath, unpredictability, absence of other working examples and lack of guidance as discussed above, undue experimentation would have been required by one skilled in the art to develop and evaluate proteinase inhibitor II nucleic acids that hybridize to SEQ ID NO: 1. Making all possible single amino acid substitutions in an 148 amino acid long protein like that encoded by SEQ ID NO: 1 would require making and analyzing 19^{148} nucleic acids; these proteins would have 99.3% identity to SEQ ID NO: 2. Because nucleic acids that hybridize to SEQ ID NO: 1 could encode proteins with many amino acid substitutions, many more than 19^{148} nucleic acids would need to be made and analyzed. Guo et al. (2004, Proc. Natl. Acad. Sci. USA 101: 9205-9210) teach that while proteins are fairly tolerant to mutations resulting in single amino acid changes, increasing the number of substitutions additively increases the probability that

the protein will be inactivated (pg 9209, right column, paragraph 2). Thus, making and analyzing proteins with many amino acid substitutions that also have hypersensitive response elicitor activity would require undue experimentation. Therefore, it would require undue experimentation to make and/or use the invention as broadly claimed.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(A.) Claim 1,3, and 49-51 are rejected under 35 U.S.C. 102(b) as being anticipated by Xu et al (Plant Molecular Biology, 2001, 47:727-738). Xu et al teach a nucleotide sequence comprising SEQ ID NO: 1 (See search results) and said sequence cloned in a vector having regulatory elements (page 729, column 1, second paragraph).

(B.) Claim 3, 11, 12, 19, 20, 33, 34, 39, 40, 49, 50, 51, 55 and 56 are rejected under 35 U.S.C. 102(b) as being anticipated by Anderson et al (29 February 2000, U.S. Patent 6,031,087). Anderson et al disclosed a nucleotide sequence that hybridizes to SEQ ID NO: 1 because it has 67.3% similarity to it (See search results). Anderson et al disclosed a method of increasing or enhancing resistance of a plant to insect or other pathogen infestation, said method comprising introducing a nucleic acid molecule into cell or group of cells of said plant, regenerating a plant therefore and growing said plant for a time and under conditions sufficient to permit expression of said nucleic acid into a

proteinase inhibitor or precursor thereof which inhibits growth and infestation by said pathogen (column 38, claim 7), a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1 (column 20, line 45), and sf9 cells infected with a recombinant virus containing the recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO: 1 (column 20, line 55) and programmed cell death would be inherent in a method having the same steps.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

(A.) Claims 1, 3 8, 9, 11, 12, 14-17, 19, 20 30, 31, 33, 34, 36, 37, 39, 40, 42, 49, 50-52 and 55-58 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xu et al (Plant Molecular Biology, 2001, 47:727-738) in view of Johnson et al (PNAS, 86:9871-9875, 1989).

Xu et al disclosed a nucleotide sequence comprising SEQ ID NO: 1, a serine proteinase inhibitor with trypsin and chymotrypsin inhibitory activities and that proteinase inhibitor II could serve an endogenous role in preventing uncontrolled proteolysis and/or a function in protecting against foreign proteolytic enzymes of pest or pathogen (Xu et al., 2001, page 727, column 1, first paragraph).

Xu et al do not teach a method for producing a transformed plant comprising transforming a plant with a polynucleotide that comprises a nucleotide sequence that hybridizes to SEQ ID NO: 1, selecting a transformed plant in which said nucleotide sequence is expressed, a transformed plant produced by the steps of transforming a plant with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence of SEQ ID NO: 1 or that hybridizes to SEQ ID NO: 1, and selecting a transformed plant in which said polynucleotide is expressed.

Johnson et al disclosed plant transformation plasmids containing either the inhibitor I or the inhibitor II coding region from the genes or cDNA, under the control of the CaMV 35S promoter (page 9872, column 2, second paragraph). Plants were transformed with these plasmids by nuclear transformation. The leaf extracts from tobacco plants transformed with the plasmids were analyzed for the expression of the proteins by assaying for the ability of the proteins to inhibit trypsin and chymotrypsin (page 9871, column 2, sixth paragraph; page 9872, column 2, third paragraph).

At the time the invention was made, it would have been obvious to one of ordinary skill in the art to modify the method for producing a transformed plant and selecting a transformed plant in which said nucleotide sequence is expressed as described by Johnson et al to substitute a recombinant vector comprising a polynucleotide that comprises the nucleotide SEQ ID NO: 1 or an isolated nucleic acid molecule having a nucleotide sequence that hybridizes to a proteinase inhibitor II nucleotide sequence of SEQ ID NO: 1. One of ordinary skill in the art would have been motivated to do so because expression of plant defense proteins in plants can enhance pest/pathogen protection in transgenic crops (Xu et al, Plant Molecular Biology, 47:727-738, 2001, page

728, column 1, second paragraph). Thus, the claimed invention would have been prima facie obvious as a whole to one of ordinary skill in the art, at the time it was made, especially in the absence of evidence to the contrary.

(B.) Claims 1, 3, 8, 10, 11, 13, 16, 18, 19, 21, 30, 32, 33, 35, 36, 38, 39, 41, 49, 50, 51, 52, 55 and 56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xu et al (Plant Molecular Biology, 2001, 47:727-738) in view of Daniell et al (U.S. Patent Application Publication No: 2004/0210966; effective filing date 02/February 2001) and Zhang et al (Plant Physiology, 127:131-141, 2001, abstract).

The teachings of Xu et al are discussed above.

Xu et al do not teach a method for producing a transformed plant comprising a polynucleotide that comprises the nucleotide sequence of SEQ ID NO: 1 or that hybridizes to SEQ ID NO: 1, a method for producing a transformed plant comprising transforming a plant with a polynucleotide that comprises SEQ ID NO: 1 or a nucleotide sequence that hybridizes to SEQ ID NO: 1, a transformed plant comprising a polynucleotide that comprises SEQ ID NO: 1or a nucleotide sequence of SEQ ID NO: via plastid transformation.

Daniell et al disclosed method for producing a transformed plant with several genes into a single T-DNA. These genes are insecticidal toxin genes such as *Bacillus thuingiensis* genes, protease inhibitors, the cowpea trypsin inhibitors, and the potato proteinase inhibitor II via plastid transformation (paragraphs 23-31; paragraph 116; claims 1, 5) and said transgenic plants produced the proteins as measured by ELISA and insect bioassays (paragraphs 131 and 132; claim 15).

At the time the invention was made, it would have been obvious to one of ordinary skill in the art to modify the method of transforming a chloroplast as taught by Daniell et al to substitute a recombinant vector comprising a polynucleotide comprising SEQ ID NO: 1 as taught by Xu et al. One of ordinary skill in the art would have been motivated to do so because very high and uniform levels of gene expression can be observed in different transplastomic lines, probably due to the identical insertion sites, in contrast to nuclear transformation where random insertion occur (Zhang et al., Plant Physiology, 127:131-141, 2001, abstract). In addition, Daniell et al recited that plants transfsormed by plastid transformation showed the highest level of expression (paragraph 8). Thus, the claimed invention would have been prima facie obvious as a whole to one of ordinary skill in the art, at the time it was made, especially in the absence of evidence to the contrary.

(C.) Claims 1,3, 16, 17, 19, 20, 22 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Solomon et al (The Plant Cell, 11:431-443, 1999) in view of Xu et al (Plant Molecular Biology, 2001, 47:727-738).

Solomon et al disclosed transformation of soybean suspension-cultured cells with a plasmid containing either a soybean cystatin (cysteine proteinase inhibitor), or Kunitz, (inhibitor of the trypsin-like proteases), or a Bowman-Birk-type inhibitor (a chymotrypsin and elastase inhibitor) operably linked to the 35S cauliflower mosaic virus promoter. These transgenic plants were analyzed for the role of specific types of proteases in soybean programmed cell death. Ectopic expression of cystatin blocked programmend cell death triggered by an avirulent strain *Pseudomonas syringae* pv

glycinea (page 435, column 2, first paragraph) or by oxidative stress (page 435, column 1, fourth paragraph).

Solomon et al do not teach a method for inhibiting programmed cell death and senescence in a transformed plant or plant part comprising transforming a plant with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes to SEQ ID NO: 1encoding SEQ ID NO: 2 and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity and selecting a transformed plant in which said polynucleotide is expressed.

Xu et al disclosed a nucleotide sequence comprising SEQ ID NO: 1, a serine proteinase inhibitor with trypsin and chymotrypsin inhibitory activities. Proteinase inhibitor II could serve an endogenous role in preventing uncontrolled proteolysis and/or a function in protecting against foreign proteolytic enzymes of pest or pathogen (Xu et al., 2001, page 727, column 1, first paragraph).

At the time the invention was made, it would have been obvious to one of ordinary skill in the art to modify the method for inhibiting programmed cell death and senescence in a transformed plant or plant part as taught by Solomon et al to substitute a polynucleotide that hybridizes to SEQ ID NO: 1 as described by Xu et al. One of ordinary skill in the art would have been motivated to do so because in order to analyze the role of specific proteases in programmed cell death. One of ordinary skill in the art would have been motivated to do so because in order to inhibit programmed cell death in plants. Thus, the claimed invention would have been prima facie obvious as a whole to one of ordinary skill in the art, at the time it was made, especially in the absence of evidence to the contrary.

(D.) Claims 1, 3, 24, 25, 27 and 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Urwin et al (Planta, 204: 472-479, 1998) in view of Xu et al (Plant Molecular Biology, 2001, 47:727-738).

Urwin et al disclosed a recombinant fusion protein expression in E. coli and analysis of inhibitory activity. The oryzacystatin (cysteine inhibitor) and cowpea trypsin inhibitor were joined as translational fusions, expressed in E. coli and the expressed protein were purified using the 6-His-tag. After the removal of the His tag peptide from the fusion protein, the protein was analyzed for its ability to inhibit the activity of papain and trypsin (page 474, column 1, third paragraph). Urwin et al also disclosed a transformed plants transformed with a plasmid containing oryzacystatin (cysteine inhibitor) and cowpea trypsin inhibitor genes in a single TDNA by nuclear transformation.

Urwin et al do not teach a transformed plants comprising transforming a plant with a polynucleotide that comprises a nucleotide sequence that hybridizes to SEQ ID NO: 1, and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity, selecting a transformed plant in which said nucleotide sequence is expressed.

The teachings of Xu et al are discussed above.

At the time the invention was made, it would have been obvious to one of ordinary skill in the art to modify the method for producing a heterologous protein in a plant described by Urwin et al to substitute a recombinant vector comprising a polynucleotide of SEQ ID NO: 1 or that hybridizes to SEQ ID NO: 1. One of ordinary skill in the art would have been motivated to do so because stacking or pyramiding transgenes enhanced efficacy and durability and thereby broadening the potential of the transgenic approach (Urwin et al., page 473, column 1, third paragraph). Thus, the claimed invention would

have been prima facie obvious as a whole to one of ordinary skill in the art, at the time it was made, especially in the absence of evidence to the contrary.

(E.) Claims 1, 3, 24, 26, 27 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Daniell et al and Zhang et al (Plant Physiology, 127:131-141, 2001, abstract) and Solomon et al (The Plant Cell, 11:431-443, 1999) in view of Xu et al (Plant Molecular Biology, 2001, 47:727-738).

The teachings of Daniell et al are discussed above.

Daniell et al do not teach a method for producing a heterologous protein in a plant comprising transforming a plant with a first polynucleotide that comprises the nucleotide sequence that hybridizes to SEQ ID NO: 1 by plastid transformation, wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity, transforming the plant with a second polynucleotide that encodes a heterologous protein and isolating said heterologous protein.

The teachings of Xu et al is discussed above.

At the time the invention was made, it would have been obvious to one of ordinary skill in the art to modify the method of transforming a chloroplast as taught by Daniell et al to substitute a recombinant vector comprising a polynucleotide that hybridizes to SEQ ID NO: 1 as taught by Xu et al and to fuse a polynucleotide that hybridizes to SEQ ID NO: 1 with His tag peptide for purification as taught bt Urwin et al. One of ordinary skill in the art would have been motivated to do so because very high and uniform levels of gene expression can be observed in different transplastomic lines, probably due to the identical insertion sites, in contrast to nuclear transformation where random insertion occur (Zhang et al., Plant Physiology, 127:131-141, 2001, abstract). In addition, Daniell

et al recited that plants transformed by plastid transformation showed the highest level of expression (paragraph 8). Thus, transplastomic plants expressing multiple proteinase inhibitors can enhance efficacy and durability and thereby broadening the potential of the transgenic approach (Urwin et al., page 473, column 1, third paragraph) and for further purification of the proteinase inhibitor II. Thus, the claimed invention would have been prima facie obvious as a whole to one of ordinary skill in the art, at the time it was made, especially in the absence of evidence to the contrary.

Claims 43-47 and 53-54 are free of prior art.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Teresa Samson whose telephone number is 571-272-3110. The examiner can normally be reached on 7:00-5:00. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson, can be reached on 571-272-0804. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public. For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

Maria Teresa Samson, Ph.D
February 22, 2005

ELIZABETH MCELWAIN
PRIMARY EXAMINER

```
Qy        131 CCACGTTCAGAAGGAAGTCCCCAAAAACCTATATGCACCAATTGTTGCTCAGGCTATAAG 190
              ||||||||||||||||||||  |||| || |||||||||| |||||  |||| |||||
Db        558 CCACGTTCAGAAGGAAGTCCGGAAAATCCGATATGCACCAACTGTTGTGCAGGTTATAAA 617

Qy        191 GGTTGCAACTATTACAGTGCTAAAGGAGATTTGATTTGTGAAGGAGAATCTGACCCTAGA 250
              |||||||| |||||  |||||  || || | ||| |||||||||||| |||||||||| | |
Db        618 GGTTGCAATTATTATAGTGCAAATGGGGCTTTCATTTGTGAAGGACAATCTGACCCAAAA 677

Qy        251 AACCCAAAAGATTGTACCTTCGAATGTGATACACAGATTGCTTATTCAAAATGTCCTCGT 310
              || |||||||  ||  || |  | || ||| |||| ||||| || ||||| ||||| |||
Db        678 AAACCAAAAGCATGCCCCCTAAATTGCGATCCACATATTGCCTACTCAAAGTGTCCCCGT 737

Qy        311 TCAGAAGGAAAGATGATAATTAAACCCACTGGATGCACCACTTGTTGCACGGGCTATCAG 370
              ||||||||||   | |||||  | |||||  |||||  |||||  || |||||  ||  ||
Db        738 TCAGAAGGAAAATCGCTAATTTATCCCACCGGATGTACCACATGCTGCACAGGGTACAAG 797

Qy        371 GGTTGCTACTATTTCGATCAAGATGGTGATTTTGTCTGTGAAGGAGAGAGTCCTGAACCC 430
              |||||||||||||||| | || ||||  | ||||| ||||||||||||||  ||| |||
Db        798 GGTTGCTACTATTTCGGTAAAAATGGCAAGTTTGTATGTGAAGGAGAGAGTGATGAGCCC 857

Qy        431 AAGACCACTGCTTA 444
              ||| | | |   ||
Db        858 AAGGCAAATATGTA 871
```

RESULT 5
AAZ49862
ID AAZ49862 standard; DNA; 584 BP.
XX
AC AAZ49862;
XX
DT 25-APR-2000 (first entry)
XX
DE Potato proteinase inhibitor-II DNA.
XX
KW Potato proteinase inhibitor-II; PPI-II; strepavidin; worm; insect;
KW plant-noxious protein; pest resistance; moth; insect; weevil; grub;
KW beetle; fly; thrip; locust; cricket; borer; mite; looper; insecticidal;
KW ss.
XX
OS Solanum tuberosum.
XX

FH	Key	Location/Qualifiers
FT	CDS	1. .584
FT		/*tag= a
FT		/product= "Potato proteinase inhibitor-II"
FT		/note= "coding region contains one intron"
FT	sig_peptide	1. .212
FT		/*tag= b
FT		/note= "signal peptide contains one intron"
FT	exon	1. .52
FT		/*tag= d
FT		/number= 1
FT	intron	53. .171
FT		/*tag= d
FT		/number= 1
FT	exon	172. .584
FT		/*tag= d
FT		/number= 2
FT	mat_peptide	213. .581
FT		/*tag= c

XX
PN WO200004049-A1.
XX
PD 27-JAN-2000.
XX
PF 15-JUL-1999; 99WO-NZ000110.
XX
PR 15-JUL-1998; 98NZ-00331002.
XX
PA (HORT-) HORTICULTURE & FOOD RES INST NEW ZEALAND.
XX
PI Christeller JT, Sutherland PW, Murray C, Markwick NP, Philip BA;
PI Malone LA, Burgess EPJ;
XX
DR WPI; 2000-171244/15.
XX
PT New chimeric polypeptide and composition comprising the polypeptide
PT useful for conferring pest resistance on plants.
XX
PS Example 3; Fig 4; 111pp; English.
XX
CC The present sequence encodes potato proteinase inhibitor-II (PPI-II).
CC This is used in the preparation of a binary vector designed to express a
CC chimeric polypeptide comprising streptavidin mature peptide, a plant -
CC noxious protein, fused to the PPI-II signal peptide. The binary vector is
CC targetted to the vacuole by PPI-II signal sequence. Transformation of
CC plant genome with the vector can produce pest resistance in plants, plant
CC derived products and stored harvest material. Pests that can be
CC controlled include, cotton bollworm, tropical army-worm, European corn -
CC borer or red mite, tobacco horn worm, loopers, rice stem borer, porina,
CC cutworms, diamondback moth, potato tuber moth, codling moth, Indian meal
CC moth, gypsy moth, argentine stem weevil, clover root weevil, grass -
CC grubs, corn rootworm, rice and wheat weevils, mealworms, flour beetles,
CC black field cricket, locusts, sawflies, Western flower thrips, Hessian
CC flies or two-spotted mite
XX
SQ Sequence 584 BP; 184 A; 94 C; 116 G; 190 T; 0 U; 0 Other;

Query Match 48.1%; Score 254.2; DB 3; Length 584;
Best Local Similarity 79.4%; Pred. No. 5.7e-66;
Matches 301; Conservative 0; Mismatches 78; Indels 0; Gaps 0;

```
Qy         66 TTGCGAAACATGTTGATGCCAAGGCTTGTACTAGAGAATGTGGTCATTTTAGCTATGGCA 125
              | | | |||||||||||||| |||   ||||||| |||||||||| || || | | ||| |
Db        193 TGGTGGAACATGTTGATGCGAAGATCTGTACTAAAGAATGTGGTAATCTTGGGTTTGGGA 252

Qy        126 TATGCCCACGTTCAGAAGGAAGTCCCCAAAAACCTATATGCACCAATTGTTGCTCAGGCT 185
              |||||||||||||||||||||||||  |||| || ||||||| ||||||||||||||||
Db        253 TATGCCCACGTTCAGAAGGAAGTCCGAAAAATCCCATATGCATCAATTGTTGCTCAGGCT 312

Qy        186 ATAAGGGTTGCAACTATTACAGTGCTAAAGGAGATTTGATTTGTGAAGGAGAATCTGACC 245
              |||||||||| || ||||| |||| |   ||   || ||||| ||||||||||||||||
Db        313 ATAAGGGTTGTAATTATTATAGTGTTTTCGGGAGATTTATTTGCGAAGGAGAATCTGACC 372

Qy        246 CTAGAAACCCAAAAGATTGTACCTTCGAATGTGATACACAGATTGCTTATTCAAAATGTC 305
              | ||||||||||| ||| || |  | |||||||||| | ||||| ||||||| ||| |
Db        373 TAAAAAACCCAAAAGCTTGCCCCCTAAATTGTGATACAAATATTGCCTATTCAAGATGCC 432

Qy        306 CTCGTTCAGAAGGAAAGATGATAATTAAACCCACTGGATGCACCACTTGTTGCACGGGCT 365
              | | ||||||||||||   | ||||| | ||||| ||||| ||||| |||||||| || |
Db        433 CCCATTCAGAAGGAAAATCGCTAATTTATCCCACCGGATGTACCACATGTTGCACAGGGT 492

Qy        366 ATCAGGGTTGCTACTATTTCGATCAAGATGGTGATTTTGTCTGTGAAGGAGAGAGTCCTG 425
              |  |||||||||||||||||| | || |||| | ||||| || ||||||||||  ||
Db        493 ACAAGGGTTGCTACTATTTCGGTAAAAATGGCAAGTTTGTATGCGAAGGAGAGAGTGATG 552

Qy        426 AACCCAAGACCACTGCTTA 444
              |||||||| | | |   ||
Db        553 AACCCAAGGCAAATATGTA 571
```

RESULT 6
AAQ68729
ID AAQ68729 standard; DNA; 1360 BP.
XX
AC AAQ68729;
XX
DT 25-MAR-2003 (revised)
DT 02-MAR-1995 (first entry)
XX
DE Full length sequence of PI precursor.
XX
KW Type II serine proteinase inhibitor precursor; PI; tobacco;
KW transgenic plant; anti-pathogen; anti-predator; ss.

```
XX
OS   Nicotiana alata.
XX
FH   Key             Location/Qualifiers
FT   misc_signal     1. .97
FT                   /*tag=  b
FT                   /label= signal sequence
FT   CDS             97. .1200
FT                   /*tag=  a
XX
PN   WO9413810-A1.
XX
PD   23-JUN-1994.
XX
PF   16-DEC-1993;   93WO-AU000659.
XX
PR   16-DEC-1992;   92AU-00006399.
XX
PA   (UYME ) UNIV MELBOURNE.
XX
PI   Anderson MA,  Atkinson AH,  Heath RL,  Clarke AE;
XX
DR   WPI; 1994-217886/26.
DR   P-PSDB; AAR54135.
XX
PT   Nicotiana alata type II serine protease inhibitor precursor and DNA -
PT   useful in prodn of anti-pathogen or anti-predator constructs for plants.
XX
PS   Disclosure; Page 45-47; 83pp; English.
XX
CC   A cDNA library, prepd. from mRNA from the stigmas and styles of mature
CC   flowers of N. alata was screened for clones of highly expressed genes
CC   which were not associated with self-incompatibility genotype. Clones
CC   encoding a protein with some identity to the type II proteinase
CC   inhibitors from potato and tomato were selected. The largest clone, NA-PI
CC   -2, is given in AAQ68729. The predicted AA sequece in AAR54135. (Updated
CC   on 25-MAR-2003 to correct PN field.)
XX
SQ   Sequence 1360 BP; 416 A; 219 C; 345 G; 380 T; 0 U; 0 Other;

  Query Match             33.8%;  Score 178.8;  DB 2;  Length 1360;
  Best Local Similarity   67.3%;  Pred. No. 3.9e-43;
  Matches  303;  Conservative    0;  Mismatches  132;  Indels   15;  Gaps    3;

Qy          5 ATGGCTGTTCACAAAGTTAGCTTCCTTGCTTGCCTACTTGTTCTTGGATGGATGTTTCTA 64
              ||||||||||||| |||||| |||||||||  ||| ||  |  |||||  |    | ||
Db         10 ATGGCTGTTCACAGAGTTAGTTTCCTTGCTCTCCTCCTCTTATTTGGAATGTCTCTGCTT 69

Qy         65 CT------TGCGAAACATGTTGATGCCAAGGCTTGTACTAGAGAATGTGGTCATT---TT 115
              ||      || | ||||||  ||||||||||||||||||  | | |||| ||      ||
Db         70 GTAAGCAATGTGGAACATGCAGATGCCAAGGCTTGTACCTTAAACTGTGATCCAAGAATT 129

Qy        116 AGCTATGGCATATGCCCACGTTCAGAAGGAAGTCCCCAAAAACCTATATGCACCAATTGT 175
              ||||||  | |||||  |||||||||| ||       |  | |  ||||||||||| |||
Db        130 GCCTATGGAGTTTGCCCGCGTTCAGAAGAAAAGAAGAATGATCGGATATGCACCAACTGT 189

Qy        176 TGCTCAGGCTATAAGGGTTGCAACTATTACAGTGCTAAAGGAGATTTGATTTGTGAAGGA 235
              ||| |||||   |||||||| || || | |||||  | | |||  |||  ||||||||||
Db        190 TGCGCAGGCACGAAGGGTTGTAAGTACTTCAGTGATGATGGAACTTTTGTTTGTGAAGGA 249

Qy        236 GAATCTGACCCTAGAAACCCAAAAGATTGTACCTTCGAATGTGATACACAGATTGCTTAT 295
              || ||||| |||||||| ||||| | |||||||||  | |||||| ||   ||||| |||
Db        250 GAGTCTGATCCTAGAAATCCAAAGGCTTGTACCTTAAACTGTGATCCAAGAATTGCCTAT 309

Qy        296 TCAAAATGTCCTCGTTCAGAAGGAAAGATGATAATTAAACCCACTGGATGCACCACTTGT 355
              |   || || |||||||||| ||||| |      ||        ||||||||  |||
Db        310 GGAGTTTGCCCGCGTTCAGAAGAAAAGAAG------AATGATCGGATATGCACCAACTGT 363

Qy        356 TGCACGGGCTATCAGGGTTGCTACTATTTCGATCAAGATGGTGATTTTGTCTGTGAAGGA 415
              ||| | |||    |||||||  | || |||  | | |||||   |||||| |||||||||
Db        364 TGCGCAGGCACGAAGGGTTGTAAGTACTTCAGTGATGATGGAACTTTTGTTTGTGAAGGA 423

Qy        416 GAGAGTCCTGAACCCAAGACCACTGCTTAT 445
```

```
              ||| | | |      || | | |||| |
Db        424 GAGTCTGATCCTAGAAATCCAAAGGCTTGT 453

RESULT 7
AAQ68728
ID   AAQ68728 standard; DNA; 1104 BP.
XX
AC   AAQ68728;
XX
DT   25-MAR-2003  (revised)
DT   02-MAR-1995  (first entry)
XX
DE   Nucleotide coding region of N-alata PI precursor.
XX
KW   Type II serine proteinase inhibitor precursor; PI; tobacco;
KW   transgenic plant; anti-pathogen; anti-predator; ss.
XX
OS   Nicotiana alata.
XX
FH   Key             Location/Qualifiers
FT   CDS             1. .1104
FT                   /*tag=  a
XX
PN   WO9413810-A1.
XX
PD   23-JUN-1994.
XX
PF   16-DEC-1993;   93WO-AU000659.
XX
PR   16-DEC-1992;   92AU-00006399.
XX
PA   (UYME ) UNIV MELBOURNE.
XX
PI   Anderson MA,  Atkinson AH,  Heath RL,  Clarke AE;
XX
DR   WPI; 1994-217886/26.
DR   P-PSDB; AAR54135.
XX
PT   Nicotiana alata type II serine protease inhibitor precursor and DNA -
PT   useful in prodn of anti-pathogen or anti-predator constructs for plants.
XX
PS   Claim 5; Page 44-45; 83pp; English.
XX
CC   A cDNA library, prepd. from mRNA from the stigmas and styles of mature
CC   flowers of N. alata was screened for clones of highly expressed genes
CC   which were not associated with self-incompatibility genotype. Clones
CC   encoding a protein with some identity to the type II proteinase
CC   inhibitors from potato and tomato were selected. The largest clone, NA-PI
CC   -2, is given in AAQ68729. The predicted AA sequece in AAR54135. AAQ68728
CC   is the coding region of AAQ68729. A nucleic acid isolate having at least
CC   55% similarity to AAQ68728 is claimed. (Updated on 25-MAR-2003 to correct
CC   PN field.)
XX
SQ   Sequence 1104 BP; 337 A; 176 C; 295 G; 296 T; 0 U; 0 Other;

  Query Match             28.2%;  Score 149;  DB 2;  Length 1104;
  Best Local Similarity   67.2%;  Pred. No. 3.3e-34;
  Matches  244;  Conservative    0;  Mismatches  110;  Indels    9;  Gaps    2;

Qy         86 AAGGCTTGTACTAGAGAATGTGGTC---ATTTTAGCTATGGCATATGCCCACGTTCAGAA 142
              ||||||||||    | | |||| ||       ||  ||||||  | ||||| |||||||||
Db          1 AAGGCTTGTACCTTAAACTGTGATCCAAGAATTGCCTATGGAGTTTGCCCGCGTTCAGAA 60

Qy        143 GGAAGTCCCCAAAAACCTATATGCACCAATTGTTGCTCAGGCTATAAGGGTTGCAACTAT 202
              | ||       |  | |  |||||||||| |||||| |||||   |||||||| || ||
Db         61 GAAAAGAAGAATGATCGGATATGCACCAACTGTTGCGCAGGCACGAAGGGTTGTAAGTAC 120

Qy        203 TACAGTGCTAAAGGAGATTTGATTTGTGAAGGAGAATCTGACCCTAGAAACCCAAAAGAT 262
              | |||||  | | |||  |||  |||||||||||||| |||||| |||||||| ||||| | |
Db        121 TTCAGTGATGATGGAACTTTTGTTTGTGAAGGAGAGTCTGATCCTAGAAATCCAAAGGCT 180

Qy        263 TGTACCTTCGAATGTGATACACAGATTGCTTATTCAAAATGTCCTCGTTCAGAAGGAAAG 322
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OM nucleic - nucleic search, using sw model

Run on: January 22, 2005, 01:01:52 ; Search time 2760 Seconds
(without alignments)
9063.859 Million cell updates/sec

Title: US-10-725-829-1
Perfect score: 529
Sequence: 1 cataatggctgttcacaaag.....ctgtaatgtggactttattg 529

Scoring table: IDENTITY_NUC
Gapop 10.0 , Gapext 1.0

Searched: 4526729 seqs, 23644849745 residues

Total number of hits satisfying chosen parameters: 9053458

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : GenEmbl:*
1: gb_ba:*
2: gb_htg:*
3: gb_in:*
4: gb_om:*
5: gb_ov:*
6: gb_pat:*
7: gb_ph:*
8: gb_pl:*
9: gb_pr:*
10: gb_ro:*
11: gb_sts:*
12: gb_sy:*
13: gb_un:*
14: gb_vi:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	529	100.0	529	8	AF174381	AF174381 Solanum a
2	288	54.4	687	8	AY422686	AY422686 Solanum n
3	288	54.4	766	8	LECEVI57G	X94946 L.esculentu
4	279.6	52.9	838	8	STPIN2W	X99095 S.tuberosum
5	265.4	50.2	660	8	TOMWIPII	K03291 Tomato leaf
6	265.4	50.2	1776	8	BT013250	BT013250 Lycopersi
7	263.6	49.8	1695	8	STPRIN2G	Z12753 S.tuberosum
8	260.6	49.3	2068	8	STU45450	U45450 Solanum tub
9	259.2	49.0	559	8	AB110700	AB110700 Lycopersi
10	259.2	49.0	684	8	AY007240	AY007240 Lycopersi
11	259.2	49.0	1670	8	AY129402	AY129402 Lycopersi
12	258	48.8	1241	8	POTIIKA	M15186 S.tuberosum
13	257.6	48.7	482	8	AY247794	AY247794 Solanum p
14	256.8	48.5	512	8	STPIIIR1	X03778 Potato (Sol
15	256.8	48.5	666	8	POTPPINHB	L37519 Solanum tub
16	256.4	48.5	1274	8	NTPROTINH	Z29537 N.tabacum (
17	255.8	48.4	2330	8	STPRINII	Z13992 S.tuberosum
18	254.2	48.1	584	8	STPIN2	X78275 S.tuberosum
19	251.8	47.6	580	8	AY517498	AY517498 Solanum p
20	250.2	47.3	1914	8	STPI2G	X04118 Potato gene
21	249	47.1	554	8	STPIIIR2	X03779 Potato (Sol
22	227.4	43.0	532	8	AF209709	AF209709 Solanum a
23	219.4	41.5	1573	8	STPRINPSG	Z12824 S.tuberosum
24	178.8	33.8	1342	8	NAU08219	U08219 Nicotiana a
25	178.8	33.8	1360	6	E54395	E54395 Transgenic
26	178.8	33.8	1360	6	AR224422	AR224422 Sequence
27	178.8	33.8	1360	6	AR230263	AR230263 Sequence
28	175.6	33.2	846	8	AF105340	AF105340 Nicotiana
29	164.4	31.1	1546	8	AF542547	AF542547 Nicotiana
30	158	29.9	1795	8	AF205851	AF205851 Nicotiana
31	149.6	28.3	764	8	AF221097	AF221097 Capsicum
32	149	28.2	1104	6	E54394	E54394 Transgenic
33	149	28.2	1104	6	AR224421	AR224421 Sequence
34	149	28.2	1104	6	AR230262	AR230262 Sequence
35	148.8	28.1	1329	8	AF205852	AF205852 Nicotiana
36	143.8	27.2	836	8	AF039398	AF039398 Capsicum
37	132.8	25.1	1414	8	AY426751	AY426751 Nicotiana
38	114.2	21.6	573	8	AF242734	AF242734 Capsicum
39	105.8	20.0	562	8	BT013127	BT013127 Lycopersi
40	93	17.6	841	8	TOMARPIX	L21194 Lycopersico
41	87.8	16.6	4272	8	TOMARPI	L25128 Lycopersico
42	66.2	12.5	175	8	AY184823	AY184823 Nicotiana
43	42.8	8.1	1379	5	CR733321	CR733321 Gallus ga
44	42.2	8.0	536	11	G58878	G58878 SHGC-105534
45	42	7.9	235502	2	AC103222	AC103222 Rattus no

ALIGNMENTS

RESULT 1
AF174381
LOCUS AF174381 529 bp mRNA linear PLN 15-JAN-2002
DEFINITION Solanum americanum proteinase inhibitor IIa (PIN2a) mRNA, complete cds.
ACCESSION AF174381
VERSION AF174381.1 GI:17221674
KEYWORDS .
SOURCE Solanum americanum
ORGANISM Solanum americanum
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; asterids; lamiids; Solanales; Solanaceae; Solanum.
REFERENCE 1 (bases 1 to 529)
AUTHORS Xu,Z.F., Qi,W.Q., Ouyang,X.Z., Yeung,E. and Chye,M.L.
TITLE A proteinase inhibitor II of Solanum americanum is expressed in phloem
JOURNAL Plant Mol. Biol. 47 (6), 727-738 (2001)
MEDLINE 21643209
PUBMED 11785934
REFERENCE 2 (bases 1 to 529)
AUTHORS Chye M.-L. and Xu Z.-F.
TITLE Direct Submission
JOURNAL Submitted (01-AUG-1999) Department of Botany, The University of Hong Kong, Pokfulam Road, Hong Kong, China
FEATURES Location/Qualifiers
source 1. .529
/organism="Solanum americanum"
/mol_type="mRNA"
/db_xref="taxon:109975"
gene 1. .529
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CDS 5. .451
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Best Local Similarity   100.0%;  Pred. No. 1.3e-130;
Matches  529;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

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RESULT 2
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DEFINITION Solanum nigrum proteinase inhibitor 2b precursor (PIN2b) mRNA, complete cds.
ACCESSION AY422686
VERSION AY422686.1 GI:40036963
KEYWORDS .
SOURCE Solanum nigrum (black nightshade)
ORGANISM Solanum nigrum
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; asterids; lamiids; Solanales; Solanaceae; Solanum.
REFERENCE 1 (bases 1 to 687)
AUTHORS Schmidt,D.D., Kessler,A., Kessler,D., Schmidt,S., Lim,M., Gase,K. and Baldwin,I.T.
TITLE Solanum nigrum: a model ecological expression system and its tools
JOURNAL Mol. Ecol. 13 (5), 981-995 (2004)
PUBMED 15078438
REFERENCE 2 (bases 1 to 687)
AUTHORS Schmidt,D.D., Gase,K. and Baldwin,I.T.
TITLE Direct Submission
JOURNAL Submitted (25-SEP-2003) Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans-Knoell-Strasse 8, Jena D-07745, Germany
FEATURES Location/Qualifiers
source 1. .687
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ORIGIN

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Qy        129 GCCCACGTTCAGAAGGAAGTCCCCAAAAACCTATATGCACCAATTGTTGCTCAGGCTATA 188
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Qy        249 GAAACCCAAAAGATTGTACCTTCGAATGTGATACACAGATTGCTTATTCAAAATGTCCTC 308
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Qy        309 GTTCAGAAGGAAAGATGATAATTAAACCCACTGGATGCACCACTTGTTGCACGGGCTATC 368
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Qy        369 AGGGTTGCTACTATTTCGATCAAGATGGTGATTTTGTCTGTGAAGGAGAGAGTCCTGAAC 428
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RESULT 3
LECEVI57G
LOCUS LECEVI57G 766 bp mRNA linear PLN 19-JUL-1996
DEFINITION L.esculentum mRNA for protein inhibitor II.
ACCESSION X94946
VERSION X94946.1 GI:1161571
KEYWORDS cevi57 gene; proteinase inhibitor II.
SOURCE Lycopersicon esculentum (tomato)
ORGANISM Lycopersicon esculentum
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; asterids; lamiids; Solanales; Solanaceae; Solanum; Lycopersicon.
REFERENCE 1
AUTHORS Gadea,J., Mayda,M.E., Conejero,V. and Vera,P.
TITLE Characterization of defense-related genes ectopically expressed in viroid-infected tomato plants
JOURNAL Mol. Plant Microbe Interact. 9 (5), 409-415 (1996)
MEDLINE 96252900
PUBMED 8672818
REFERENCE 2 (bases 1 to 766)